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THE MUTAGENIC POTENTIAL OF: N-(N-OCTYL)-GLUTARIMIDE.(U)  
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INSTITUTE REPORT NO. 97V

THE MUTAGENIC POTENTIAL OF: n-(n-octyl)-glutarimide

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FREDDICA R. PULLIAM, BS, SSG  
and  
JOHN T. FRUIN, DVM, PhD, LTC VC

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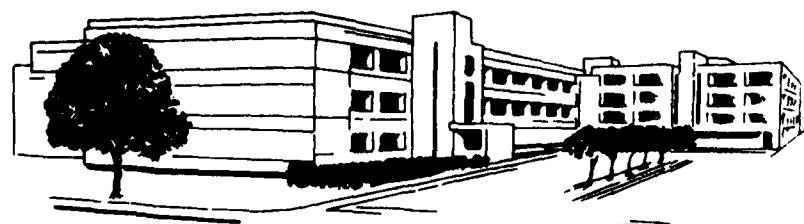
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| 20. ABSTRACT (Continue on reverse side if necessary and identify by block number)<br><br>It has been shown that with the Ames Test, N-(n-octyl)-glutarimide is not mutagenic. The assay was conducted using tester strains TA 98, TA 100, TA 1535, TA 1537, and TA 1538 at dilutions of 0.0001 ml/plate to $3.2 \times 10^{-8}$ ml/plate. |   |   |

#### ABSTRACT

It has been shown that with the Ames Assay, N-(n-octyl)-glutarimide is not mutagenic. The assay was conducted using tester strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 at dilutions of 0.0001 ml/plate to  $3.2 \times 10^{-8}$  ml/plate.

PREFACE

AMES ASSAY REPORT: N-(n-octyl)-glutarimide

TESTING FACILITY: Letterman Army Institute of Research  
Presidio of San Francisco, CA 94129

SPONSOR: Division of Cutaneous Hazards  
Letterman Army Institute of Research

PROJECT: More Effective Topical Repellents Against Disease Bearing  
Mosquitoes 3M62272A810

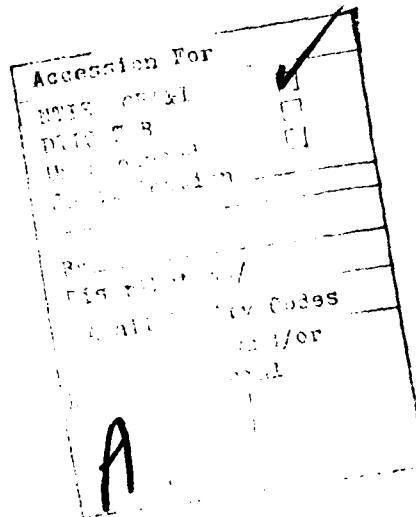
GLP STUDY NUMBER: 80007

STUDY DIRECTOR: LTC John T. Fruin, D.V.M., PhD

PRINCIPAL INVESTIGATOR: SSG Freddica R. Pulliam, BS

RAW DATA: A copy of the final report, study protocol, and retired  
SOPs will be retained in the LAIR Archives. Test compounds  
were provided by the sponsor. Chemical, analytical,  
stability, purity, etc. data are available  
from sponsor.

PURPOSE: To determine the mutagenic potential of N-(n-octyl)-  
glutarimide by using the Ames Salmonella/Mammalian  
Microsome Mutagenicity Test. Tester strains TA 98,  
TA 100, TA 1535, TA 1537 and TA 1538 were used.



#### **ACKNOWLEDGMENTS**

The authors wish to thank Ms. Carolyn Lewis, SP5 Robert Summers,  
and SP4 Thomas Kellner for their assistance in performing the  
research.

Signatures of Principal Scientists Involved  
In The Study

We, the undersigned, believe the study described in this report to be scientifically sound and the results and interpretation to be valid. The study was conducted to comply to the best of our ability with the Good Laboratory Practice Regulations outlined by the Environmental Protection Agency.

  
FREDDICA R. PULLIAM  
SSG, BS  
Principal Investigator

  
JOHN T. FRUIN, DVM, PhD  
LTC, VC  
Study Director



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REPLY TO  
ATTENTION OF:

SGRD-ULZ-QA

8 January 1981

MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance

I hereby certify that in relation to LAIR GLP study 80007 the following inspections were made:

9 June 1980  
7 July 1980

Findings were reported to the Study Director and laboratory management on 7 August 1980. Routine inspections with no adverse findings are reported quarterly, thus these inspections are also included in the July 1980 and October 1980 reports to management and the Study Director.

A handwritten signature in black ink, appearing to read "John L. Szurek".

JOHN L. SZUREK  
MAJ, MS  
Quality Assurance Officer

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### Rationale for using the Ames Assay

The Ames Salmonella/Mammalian Microsome Mutagenicity Test is one of a standard bank of tests used by our laboratory for the assessment of the mutagenic potential of a test substance. It is a short-term screening assay for the prediction of potential mutagenic agents in mammals. It is inexpensive when compared to in vivo tests, yet is highly predictive and reliable in its ability to detect mutagenic activity and therefore carcinogenic probability (1). It relies on basic genetic principles and allows for the incorporation of a mammalian microsome enzyme system to increase sensitivity through enzymatically altering the test substance into an active metabolite. It has proven highly effective in assessing human risk (1).

### Description of Test (Rationale for the selection of strains)

The test was developed by Bruce Ames, Ph.D. from the University of California-Berkeley. The test involves the use of several different genetically altered strains of Salmonella typhimurium, each with a specific mutation in the histidine operon (2). The test substance demonstrates mutagenic potential if it is able to revert the mutation in the bacterial histidine operon back to the wild type and thus reestablish prototrophic growth within the test strain. This reversion also can occur spontaneously due to a random mutational event. If, after adding a test substance, the number of revertants is significantly greater than the spontaneous reversion rate, then the test substance physically altered the locus involved in the operon's mutation and is able to induce point mutations and genetic damage (2).

In order to increase the sensitivity of the test system, two other mutations in the Salmonella are used (2). To insure a higher probability of uptake of test substance, the genome for the lipopolysaccharide layer (LP) is mutated and allows larger molecules to enter the bacteria. Each strain has another induced mutation which causes loss of excision repair mechanisms. Since many chemicals are not by themselves mutagenic but have to be activated by an enzymatic process, a mammalian microsome system is incorporated. These microsomal enzymes are obtained from livers of rats induced with Aroclor 1254; the enzymes allow for the expression of the metabolites in the mammalian system. This activated rat liver microsomal enzyme homogenate is termed S-9.

Description of Strains (History of the strains used, methods to monitor the integrity of the organisms, and data pertaining to current and historical controls and spontaneous reversion rates)

The test consists of using five different strains of *Salmonella typhimurium* that are unable to grow in absence of histidine because of a specific mutation in the histidine operon. This histidine requirement is verified by attempting to grow the tester strains on minimal glucose agar (MGA) plates, both with and without histidine. The dependence on this amino acid is shown when growth occurs only in its presence. The plasmids in strains T<sub>1</sub>, T<sub>9</sub>, and T<sub>10</sub> contain an ampicillin resistant R factor. Strains deficient in this plasmid demonstrate a zone of growth inhibition around an ampicillin impregnated disc. The alteration of the LP layer allows uptake by the *Salmonella* of larger molecules. If a crystal violet impregnated disc is placed onto a plate containing any one of the bacterial strains, a zone of growth inhibition will occur because the LP layer is altered. The absence of excision repair mechanisms can be determined by using ultraviolet (UV) light. These mechanisms function primarily by repairing photodimers between pyrimidine bases; exposure of bacteria to UV light will activate the formation of these dimers and cause cell lethality, since excision of these photodimers can not be made. The genetic mutation resulting in UV sensitivity also induces a dependence by the *Salmonella* to biotin. Therefore, this vitamin must be added. In order to prove that the bacteria are responsive to the mutation process, positive controls are run with known mutagens. If after exposure to the positive control substance, a larger number of revertants are obtained, then the bacteria are adequately responsive. Sterility controls are performed to determine the presence of contamination. Sterility of the test compound is also confirmed in each first dilution. Verification of the tester strains occurs spontaneously with the running of each assay. The value of the spontaneous reversion rate is obtained using the same inoculum of bacteria that is used in the assay (3).

Strains were obtained directly from Dr. Ames, University of California, Berkeley, propagated and then maintained at -80 C in our laboratory. Before any substance was tested, quality controls were run on the bacterial strains to establish the validity of their special features and also to determine the spontaneous reversion rate (2). Records are maintained of all the data, to determine if deviations from the set trends have occurred.

We compared the spontaneous reversion values with our own historical values and those cited by Ames et al (2). Our conclusions are based on the spontaneous reversion rate compared to the experimentally induced rate of mutation. When operating effectively, these strains detect substances that cause base pair

mutations (TA 1535, TA 100) and frameshift mutations (TA 1537, TA 1538 and TA 98) (2).

### METHODS (3)

#### Rationale for Dosage Levels and Dose Response Tabulations

To insure readable and reliable results, a sublethal concentration of the test substance had to be determined. This toxicity level was found by using MGA plates, various concentrations of the substance, and approximately  $10^8$  cells of TA 100 per plate, unless otherwise specified. Top agar containing trace amounts of histidine and biotin were placed on MGA plates. TA 100 is used because it is the most sensitive strain. Strain verification was confirmed on the bacteria, along with a determination of the spontaneous reversion rate. After incubation, the growth was observed on the plates. (The auxotrophic *Salmonella* will replicate a few times and potentially express a mutation. When the histidine and biotin supplies are exhausted, only those bacteria that reverted to the prototrophic phenotype will continue to reproduce and form macro-colonies; the remainder of the bacteria comprises the background lawn. The minimum toxic level is defined as the lowest serial dilution at which decreased macrocolony formation, below that of the spontaneous revertant rate, and an observable reduction in the density of the background lawn occurs.) A maximum dose of 1 mg/plate is used when no toxicity is observed. The densities were recorded as normal slight, and no growth.

#### Test Format

After we validated our bacterial strains and determined the optimal dosage of the test substance, we began the Ames Assay. In the actual experiment, 0.1ml of the particular strain of *Salmonella* ( $10^8$  cells) and the specific dilutions of the test substance were added to 2 ml of molten top agar, which contained trace amounts of histidine and biotin. Since survival is better from cultures which have just passed the log phase, the *Salmonella* strains were used 16 hours (maximum) after initial inoculation into nutrient broth. The dose of the test substance spanned more than a 1000-fold, decreasing from the minimum toxic level by a dilution factor of 5. All the substances were tested with and without S-9 microsome fraction. The S-9 mixture which was previously titered at an optimal strength was added to the molten top agar. After all the ingredients were added, the top agar was vortexed, then overlayed on minimum glucose agar plates. These plates contained 2% glucose and Vogel-Borner "E" Concentrate (4). The water used in this medium and all reagents came from a polystyrene system. Plates were incubated, upside down in the dark at 37 C for 48 hours. Plates were prepared in triplicate and the average revertant counts were recorded. The corresponding number of revertants obtained was compared to the number of spontaneous

revertants; the conclusions were recorded statistically. A correlated dose response is considered necessary to declare a substance as a mutagen. Commoner (5), in his report, "Reliability of Bacterial Mutagenesis Techniques to Distinguish Carcinogenic and Non-Carcinogenic Chemical," and McCann et al (1) in their paper, "Detection of Carcinogens as Mutagen: Assay of over 300 Chemicals," have concurred on the test's ability to detect mutagenic potential.

#### Statistical Analysis

Quantitative evaluation was ascertained by two independent methods. Ames et al (2) assumed that a compound which caused twice the spontaneous reversion rate is mutagenic. Commoner (5), developed the MUTAR Ratio, which is stated in the following equation:

$$\text{MUTAR} = (E - C)/C_{AV}$$

Here,  $C$  is the number of spontaneous revertant colonies on control plates obtained on the same day and with the same treatment and strains.  $E$  is the number of revertants in response to the compound;  $C_{AV}$  is the number of spontaneous revertants on control plates calculated from historical records. The explanation of the results of this equation can be determined by the method of Commoner (5). This variation determines the probability of correctly classifying substances as carcinogens on the basis of their mutagenic activity. The  $E$  values were recorded by strain, with and without S-9. Values for  $C$  and  $C_{AV}$  were recorded separately.

We used the formula and logged all values for our permanent records.

#### RESULTS

On 10 June 1980, our laboratory conducted an Ames Assay on N-(n-octyl) - glutarimide and observed toxicity in the initial dilutions of  $1 \times 10^{-3}$  and  $2 \times 10^{-4}$  ml/plate. We also observe isolated incidences of toxicity at the  $1.6 \times 10^{-6}$  ml/plate concentration. Consequently, we decided to run another assay using  $1 \times 10^{-4}$  ml/plate as the initial dose. This assay was run on 7 July 1980. We could not draw conclusive results from this second assay because there were several scattered incidences of mutagenic activity. To verify our results, we performed the test again on 11 December 1980.

In the experiment performed on 10 June 1980, spontaneous reversion values were below those suggested by Ames et al (2) for activated TA 98, TA 100 and TA 1535 and nonactivated TA 98, TA 100, TA 1535 and TA 1538 (Table 1A). On 7 July 1980, the spontaneous reversion values were below those suggested by Ames et al (2) for TA 1535 and TA 100 when activated. The results were the same for nonactivated TA 98, TA 100, and TA 1535, and TA 1538 (Table 1B). On

11 December 1980 the reversion values were below suggested levels for activated and nonactivated TA 98 and TA 100 and nonactivated TA 1538 (Table 1C). The spontaneous reversion values below those suggested by Ames et al (2) are indicative of high quality water, materials, techniques, etc; whereas, levels above the suggested range are indicators of serious assay performance problems. All the sterility and quality controls were normal for all the assays (Table 1A-1C). The positive controls were normal on 10 June 1980 and 7 July 1980 (Table 2A-B). TA 98 and TA 1538 did not respond as expected to positive control chemical dimethyl benzanthracene (DMBA) on 11 December 1980 (Table 2C). Our data are still valid because these two strains responded to aminofluorene (AF) and benzo( $\alpha$ )pyrene (BP) which function similarly (Table 2C). The toxicity test was performed on 2 May 1980 (Table 3). In that assay, the sublethal dose was determined to be  $1 \times 10^{-4}$  ml/plate (Table 4).

#### DISCUSSION

While surveying the mutagenic potential of N-(n-octyl)-glutarimide, the initial results were inconclusive. The assay of 10 June 1980 and 7 July 1980 demonstrated isolated incidences of mutagenic activity. On 11 December 1980, we decided to perform the assay again. On 10 June 1980, twice the number of revertants were yielded experimentally, as were demonstrated spontaneously for activated TA 1538 at the  $1.6 \times 10^{-6}$  dose (Table 5A). For the assay of 7 July 1980, mutagenic activity for the nonactivated TA 1535 was determined to be  $1.6 \times 10^{-7}$  ml/plate level and activated TA 1535 at the  $4 \times 10^{-6}$  and  $3.2 \times 10^{-6}$  ml/plate dose levels. The same was observed for nonactivated TA 1538 at the  $2 \times 10^{-4}$  ml/plate doses (Table 5B). On 11 Dec 80, only a numerical suggestion of mutagenicity for activated TA 1535 at the  $2 \times 10^{-5}$  and  $3.2 \times 10^{-5}$  ml/plate dose levels was observed (Table 5C). Our MUTAR values (Table 6A-6C) are well below the necessary 1.5 level needed to declare a substance mutagenic. Only activated TA 1538 at the  $1.6 \times 10^{-6}$  ml/plate dose on 10 June 1980 demonstrated a value greater than 1.5.

#### CONCLUSION

In order for a substance to be mutagenic according to the Ames Test, two criteria must be met. There must be two times the number of experimental revertants as spontaneous revertants; and, an obvious dose response must be evident. There only two isolated incidences of twice the spontaneous reversion rate, therefore, N-(n-octyl)-glutarimide does not appear to function as a mutagen.

#### RECOMMENDATION

We recommend that N-(n-octyl)-glutarimide be tested using other test systems if efficacy tests show this chemical to be a promising repellent.

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TABLE - 1 A

QUALITY CONTROL OF TESTER STRAINS WORKSHEET  
Salmonella/Microsome Assay

10 June 80

| Strain No. | Histidine (a)<br>Requirements | Ampicillin (b)<br>Resistance | uvr-B (c)<br>Deletion | rrt Crystal<br>Violet | Sensitivity<br>Control (e) |
|------------|-------------------------------|------------------------------|-----------------------|-----------------------|----------------------------|
| TA 98      | +                             | +                            | +                     | 13.35                 | NT                         |
| TA 100     | +                             | +                            | +                     | 14.72                 | NT                         |
| TA 1535    | +                             | NA                           | +                     | 15.35                 | NT                         |
| TA 1537    | +                             | 23.75                        | +                     | 14.12                 | NT                         |
| TA 1538    | +                             | NA                           | +                     | 14.58                 | NT                         |
| WT         | GROWTH                        | NA                           | GROWTH                | NA                    | NA                         |

QUALITY CONTROL (e)

His-Bio mix      Initial: NT      End: NT      Test Compound 1: NC  
 Top Agar      Initial: NT      End: NT      Test Compound 2: NA  
 S - 9      Initial: NT      End: NT      Test Compound 3: NA  
 Diluent: +      Nutrient Broth: +      Test Compound 4: NA  
 MGA Plate w/ bacteria: +      MGA Plate: +      Test Compound 5: NA

(a) + = no growth (requires histidine for growth); (b) + = no zone of inhibition, - = zone of inhibition of approximately 16mm; (c) + = no growth on irradiated side of plate; (d) + = zone of inhibition approximately 14mm diameter; (e) + = no growth (growth indicates contamination); NT=not tested; NA=no growth; WT=wild type.

## Spontaneous Revertants (1)

| Strain<br>(1) | Avg | Range   | No S-9 |     |     | Avg | S-9 |    |    | Avg |
|---------------|-----|---------|--------|-----|-----|-----|-----|----|----|-----|
| TA 98         | 40  | 30-50   | 21     | 23  | 17  | 20  | 19  | 26 | 25 | 23  |
| TA 100        | 160 | 120-200 | 99     | 121 | 116 | 112 | 120 | 67 | 76 | 83  |
| TA 1535       | 20  | 10-35   | 10     | 9   | 6   | 8   | 6   | 4  | 4  | 5   |
| TA 1537       | 7   | 3-15    | 9      | 5   | 9   | 8   | 5   | 11 | 10 | 9   |
| TA 1538       | 25  | 15-35   | 8      | 11  | 6   | 9   | 18  | 21 | 15 | 18  |

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Test Inoculated By: White, Summers, Pulliam Date: 10 June 80Test Read By: Pulliam Date: 11 June 80

TABLE - 1B  
QUALITY CONTROL OF TESTER STRAINS WORKSHEET  
Salmonella/Microsome Assay

7 July 80

| Strain No. | Histidine (a)<br>Requirements | Ampicillin (D)<br>Resistance | uvr-B (c)<br>Deletion | rfa Crystal<br>Viability | Sterility<br>Control (e) |
|------------|-------------------------------|------------------------------|-----------------------|--------------------------|--------------------------|
| TA 98      | +                             | +                            | +                     | 13.68                    | NT                       |
| TA 100     | +                             | +                            | +                     | 14.58                    | NT                       |
| TA 1535 *  | +                             | NA                           | +                     | 13.44                    | NT                       |
| TA 1537    | +                             | 25.89                        | +                     | 19.32                    | NT                       |
| TA 1538    | +                             | NA                           | +                     | 14.20                    | NT                       |
| WT         | GROWTH                        | NA                           | GROWTH                | NA                       | NA                       |

QUALITY CONTROL (e)

|                        |             |                   |                     |
|------------------------|-------------|-------------------|---------------------|
| His-Bio mix            | Initial: NT | End: NT           | Test Compound 1: MG |
| Top Agar               | Initial: NT | End: NT           | Test Compound 2: NA |
| S - 9                  | Initial: NT | End: NT           | Test Compound 3: NA |
| Diluent:               | +           | Nutrient Broth: + | Test Compound 4: NA |
| MGA Plate w/ bacteria: | +           | MGA Plate: +      | Test Compound 5: NA |

(a) + = no growth (requires histidine for growth); (b) + = no zone of inhibition,  
- = zone of inhibition of approximately 16mm; (c) + = no growth on irradiated  
side of plate; (d) + = zone of inhibition approximately 14mm diameter; (e) + = no  
growth (growth indicates contamination); NT=not tested; MG=no growth; WT=wild type.

Spontaneous Revertants (1) \* growth of TA 1535 did not  
equal that of other tubes of culture

| Strain<br>(1) | Avg | Range   | No S-9   | Avg | S-9 |    |     | Avg |
|---------------|-----|---------|----------|-----|-----|----|-----|-----|
| TA 98         | 40  | 30-50   | 16 15 24 | 18  | 30  | 35 | 31  | 32  |
| TA 100        | 160 | 120-200 | 99 44 52 | 65  | 122 | 98 | 112 | 111 |
| TA 1535       | 20  | 10-35   | 12 10 1  | 8   | 4   | 10 | 8   | 7   |
| TA 1537       | 7   | 3-15    | 4 7 0    | 4   | 7   | 9  | 8   | 8   |
| TA 1538       | 25  | 15-35   | 7 6 4    | 6   | 16  | 15 | 15  | 15  |

Ames, B.N., J. McCann and E. Yamasaki. Mutat. Res. 31:347

Test Inoculated By: F. Pulliam Date: 7 July 80

Test Read By: F. Pulliam Date: 8 July 80

TABLE - 1C  
QUALITY CONTROL OF TESTER STRAINS WORKSHEET  
Salmonella/Microsome Assay

11 Dec 80

| Strain No. | Histidine (a)<br>Requirements | Ampicillin (b)<br>Resistance | uvr-B (c)<br>Deletion | rfa Crystal<br>Violet | Sterility<br>Control (d) |
|------------|-------------------------------|------------------------------|-----------------------|-----------------------|--------------------------|
| TA 98      | +                             | +                            | +                     | 15.74mm               | NG                       |
| TA 100     | 4 colonies +                  | +                            | +                     | 15.42mm               | NG                       |
| TA 1535    | +                             | NA                           | +                     | 16.19mm               | NG                       |
| TA 1537    | 1 colony +                    | 25.41                        | +                     | 15.58mm               | NG                       |
| TA 1538    | 4 colonies +                  | NA                           | +                     | 15.63mm               | NG                       |
| WT         | GROWTH                        | NA                           | GROWTH                | NA                    | NA                       |

QUALITY CONTROL (e)

|                          |            |                   |                     |
|--------------------------|------------|-------------------|---------------------|
| His-Bio mix              | Initial: + | End: +            | Test Compound 1: NG |
| Top Agar                 | Initial: + | End: +            | Test Compound 2: NG |
| S - 9                    | Initial: + | End: +            | Test Compound 3: NA |
| Diluent: ETOH + DMSO +   |            | Nutrient Broth: + | Test Compound 4: NA |
| MGA Plate w/ bacteria: + |            | MGA Plate: +      | Test Compound 5: NA |

(a) + = no growth (requires histidine for growth); (b) + = no zone of inhibition, - = zone of inhibition of approximately 16mm; (c) + = no growth on irradiated side of plate; (d) + = zone of inhibition approximately 14mm diameter; (e) + = no growth (growth indicates contamination); NT=not tested; NG=no growth; WT=wild type.

Spontaneous Revertants (f)

| Strain<br>(1) | Avg | Range   | No S-9 |    |     | Avg | S-9 |     |     | Avg |
|---------------|-----|---------|--------|----|-----|-----|-----|-----|-----|-----|
| TA 98         | 40  | 30-50   | 19     | 16 | 15  | 17  | 26  | 22  | 16  | 21  |
| TA 100        | 160 | 120-200 | 111    | 98 | 123 | 111 | 101 | 111 | 116 | 109 |
| TA 1535       | 20  | 10-35   | 24     | 20 | 10  | 18  | 11  | 13  | 9   | 11  |
| TA 1537       | 7   | 3-15    | 5      | 7  | 8   | 7   | 6   | 7   | 8   | 7   |
| TA 1538       | 25  | 15-35   | 5      | 16 | 14  | 12  | 12  | 17  | 17  | 15  |

Ames, B.N., J. McCann and E. Yamasaki. Mutat. Res. 31:347

Sauers, Pulliam  
Test Inoculated By: Summers, Kellner Date: 11 Dec 80  
Test Read By: Sauers Date: 11 Dec 80

TABLE 2-A

POSITIVE CONTROL REVERTANT RATE

(a) + = expected result, - = unexpected result (see discipline note)

**TABLE 2-B**

(a) + = expected result, - = unexpected result (see discipline note)

TABLE 2-C

(a) + = expected result, - = unexpected result (see flexibility note)

TA 93, TA 100, and TA 1533 showed an unexpected low response to DIBA.

TABLE - 3  
STRAIN VERIFICATION FOR TOXICITY LEVEL DETERMINATION  
Salmonella/Microsome Assay

| Strain No.  | Histidine (a)<br>Requirements | Ampicillian (b)<br>Resistance | uvr=B (c)<br>Deletion | rfa Crystal<br>Violet (d) | Sterility/<br>Control (e) |
|---|-------------------------------|-------------------------------|-----------------------|---------------------------|---------------------------|
| TA 100  | +                             | +                             | +                     | 16mm                      | +                         |
| TA 1537   | +                             | 21mm                          | +                     | 16mm                      | +                         |
| WT  | NT                            | NT                            | NT                    | NT                        | NT                        |
| Diluent   | NT                            | NT                            | NT                    | NT                        | NT                        |
| Test<br>Compound (s)  |                               |                               |                       |                           |                           |
| #1 N-Hexyl-2-<br>oxazolidone  | NT                            | NT                            | NT                    | NT                        | +                         |
| #2 N-octyl-<br>glutarimide  | NT                            | NT                            | NT                    | NT                        | +                         |
| #3 _____  | NT                            | NT                            | NT                    | NT                        |                           |
| #4 _____  | NT                            | NT                            | NT                    | NT                        |                           |
| #5 _____  | NT                            | NT                            | NT                    | NT                        |                           |
| (a) + = no growth (requires histidine for growth); (b) + = no zone of inhibition,<br>- = zone of inhibition of approximately 16mm; (c) + = no growth on irradiated<br>side of plate; (d) + = zone of inhibition approximately 14mm diameter; (e) + =<br>no growth (growth indicates contamination); NT=not tested; WT= wild type. |                               |                               |                       |                           |                           |

Spontaneous Revertants

| Strain | Average | Range   |              |          |          | Average  |
|--------|---------|---------|--------------|----------|----------|----------|
| TA 100 | 160     | 120-200 | S-9<br>NOS-9 | 99<br>93 | 81<br>77 | 77<br>94 |

Test Inoculated By: F. Pulliam Date: 2 May 1980

Test Read By: F. Pulliam Date: 4 May 1980

TABLE - 4

**TOXICITY LEVEL DETERMINATION  
Salmonella/Microsome Assay**

Substance assayed: (1) N-octyl-glutarimide (2) \_\_\_\_\_  
(3) \_\_\_\_\_ (4) \_\_\_\_\_ (5) \_\_\_\_\_  
Date: 2 May 1980 Performed by: Pulliam

Substance dissolved in: (1) ETOH (2) \_\_\_\_\_ (3) \_\_\_\_\_

(4) \_\_\_\_\_ (5) \_\_\_\_\_ Visual estimation of background lawn on Nutrient Agar Plates: NG = no growth  
ST = slight growth  
NL = normal growth

**TABLE - 5A**  
**SALMONELLA/MICROSOME ASSAY WORKSHEET**  
**(POSITIVE CONTROLS/TEST COMPOUND)**

Substance Assayed: (1) octyl glutarimide (2) \_\_\_\_\_

(3) \_\_\_\_\_ (4) \_\_\_\_\_ (5) \_\_\_\_\_

Date: 10 June 80 Performed By: Pulliam, Sayers, Summers

Substance dissolved in: (1) ETOH (2)

(3) \_\_\_\_\_ (4) \_\_\_\_\_ (5) \_\_\_\_\_.

# Revertant/Plate

TABLE - 5B

**SALMONELLA/MICROSOME ASSAY WORKSHEET  
(POSITIVE CONTROLS/TEST COMPOUND)**

Substance Assayed: (1) octyl-glutarimide (2) \_\_\_\_\_

(3) \_\_\_\_\_ (4) \_\_\_\_\_ (5) \_\_\_\_\_

Date: 7 July 1980 Performed By: Pulliam, Sauers, Kellner, Summers

Substance dissolved in: (1) ETOH (2) \_\_\_\_\_

(3) \_\_\_\_\_ (4) \_\_\_\_\_ (5) \_\_\_\_\_.

# Revertant/Plate

TABLE - 5C

SALMONELLA/MICROSOME ASSAY WORKSHEET  
(POSITIVE CONTROLS/TEST COMPOUND)

Substance Assayed: (1) octyl glutarimide (2) \_\_\_\_\_  
(3) \_\_\_\_\_ (4) \_\_\_\_\_ (5) \_\_\_\_\_

Date: 11 Dec 80 Performed By: Sauers, Pulliam, Kellner, Summers

Substance dissolved in: (1) ETOH (2) \_\_\_\_\_  
(3) \_\_\_\_\_ (4) \_\_\_\_\_ (5) \_\_\_\_\_

TABLE - 6A

MUTAGENIC ACTIVITY RATIO  
Salmonella/Microsome Assay

Substance Assayed: N-octyl-glutarimide Dissolved in: ETOH  
 Date: 10 June 1980 Performed by: Pulliam

| Concentration        | Strain  | MUTAR | MUTAR act | Concentration        | Strain  | MUTAR | MUTAR act |
|----------------------|---------|-------|-----------|----------------------|---------|-------|-----------|
| 0.001                | TA 98   | *     | *         | $8 \times 10^{-6}$   | TA 1535 | *     | *         |
| $2 \times 10^{-4}$   | TA 98   | *     | *         | $1.6 \times 10^{-6}$ | TA 1535 | *     | *         |
| $4 \times 10^{-5}$   | TA 98   | *     | *         | $3.2 \times 10^{-7}$ | TA 1535 | *     | *         |
| $8 \times 10^{-6}$   | TA 98   | *     | 0.19      |                      |         |       |           |
| $1.6 \times 10^{-6}$ | TA 98   | *     | *         | 0.001                | TA 1537 | *     | *         |
| $3.2 \times 10^{-7}$ | TA 98   | 0.52  | 0.31      | $2 \times 10^{-4}$   | TA 1537 | *     | *         |
|                      |         |       |           | $2 \times 10^{-5}$   | TA 1537 | 0.16  | *         |
| 0.001                | TA 100  | *     | *         | $8 \times 10^{-6}$   | TA 1537 | *     | *         |
| $2 \times 10^{-4}$   | TA 100  | *     | *         | $1.6 \times 10^{-6}$ | TA 1537 | *     | *         |
| $4 \times 10^{-5}$   | TA 100  | *     | *         | $3.2 \times 10^{-7}$ | TA 1537 | 0.49  | 0.27      |
| $8 \times 10^{-6}$   | TA 100  | *     | *         |                      |         |       |           |
| $1.6 \times 10^{-6}$ | TA 100  | *     | *         | 0.001                | TA 1538 | *     | *         |
| $3.2 \times 10^{-7}$ | TA 100  | *     | 0.10      | $2 \times 10^{-4}$   | TA 1538 | *     | *         |
|                      |         |       |           | $4 \times 10^{-5}$   | TA 1538 | 0.12  | *         |
| 0.001                | TA 1535 | *     | *         | $8 \times 10^{-6}$   | TA 1538 | *     | *         |
| $2 \times 10^{-4}$   | TA 1535 | *     | *         | $1.6 \times 10^{-6}$ | TA 1538 | *     | 1.93      |
| $4 \times 10^{-5}$   | TA 1535 | *     | 0.21      | $3.2 \times 10^{-7}$ | TA 1538 | 0.24  | *         |

\* Calculated value results in a negative MUTAR

TABLE - 6B  
MUTAGENIC ACTIVITY RATIO  
Salmonella/Microsome Assay

Substance Assayed: N-octyl-glutarimide Dissolved in: ETOH  
Date: 7 July 1980 Performed by: Pulliam

| Concentration        | Strain  | MUTAR | MUTAR act | Concentration        | Strain  | MUTAR | MUTAR act* |
|----------------------|---------|-------|-----------|----------------------|---------|-------|------------|
| 0.0001               | TA 98   | 0.04  | 0.04      | $8 \times 10^{-7}$   | TA 1535 | *     | 0.43       |
| $2 \times 10^{-5}$   | TA 98   | 0.61  | 0.23      | $1.6 \times 10^{-7}$ | TA 1535 | 0.9   | 0.3?       |
| $4 \times 10^{-6}$   | TA 98   | 0.48  | *         | $3.2 \times 10^{-8}$ | TA 1535 | 0.08  | 1.28       |
| $8 \times 10^{-7}$   | TA 98   | 0.43  | *         |                      |         |       |            |
| $1.6 \times 10^{-7}$ | TA 98   | 0.17  | *         | 0.0001               | TA 1537 | *     | *          |
| $3.2 \times 10^{-8}$ | TA 98   | 0.22  | *         | $2 \times 10^{-5}$   | TA 1537 | *     | 0.27       |
|                      |         |       |           | $4 \times 10^{-6}$   | TA 1537 | 0.33  | *          |
| 0.0001               | TA 100  | 0.20  | *         | $8 \times 10^{-7}$   | TA 1537 | 0.66  | *          |
| $2 \times 10^{-5}$   | TA 100  | 0.35  | *         | $1.6 \times 10^{-7}$ | TA 1537 | 0.33  | *          |
| $4 \times 10^{-6}$   | TA 100  | 0.33  | 0.32      | $3.2 \times 10^{-8}$ | TA 1537 | 0.49  | *          |
| $8 \times 10^{-7}$   | TA 100  | 0.41  | *         |                      |         |       |            |
| $1.6 \times 10^{-7}$ | TA 100  | 0.34  | 0.14      | 0.0001               | TA 1538 | 0.12  | 0.17       |
| $3.2 \times 10^{-8}$ | TA 100  | 0.34  | *         | $2 \times 10^{-5}$   | TA 1538 | 0.84  | *          |
|                      |         |       |           | $4 \times 10^{-6}$   | TA 1538 | 0.48  | 0.17       |
| 0.0001               | TA 1539 | 0.38  | 0.11      | $8 \times 10^{-7}$   | TA 1538 | *     | 0.12       |
| $2 \times 10^{-5}$   | TA 1539 | *     | 0.53      | $1.6 \times 10^{-7}$ | TA 1538 | 0.36  | 0.23       |
| $4 \times 10^{-6}$   | TA 1539 | *     | 1.28      | $3.2 \times 10^{-8}$ | TA 1538 | *     | *          |

\* Calculated value resulted in a negative MUTAR

TABLE - 6C

MUTAGENIC ACTIVITY RATIO  
Salmonella/Microsome AssaySubstance Assayed: octyl glutarimide Dissolved in: ETOHDate: 11 Dec 80 Performed by: Sauers, Pulliam

| Concentration        | Strain  | MUTAR | MUTAR act | Concentration        | Strain  | MUTAR | MUTAR act |
|----------------------|---------|-------|-----------|----------------------|---------|-------|-----------|
| 0.0001               | TA 98   | 0.13  | 0.04      | $8 \times 10^{-7}$   | TA 1535 | *     | 1.06      |
| $2 \times 10^{-5}$   | TA 98   | 0.13  | 0.08      | $1.6 \times 10^{-7}$ | TA 1535 | 0.45  | 0.74      |
| $4 \times 10^{-6}$   | TA 98   | 0.17  | 0.19      | $3.2 \times 10^{-8}$ | TA 1535 | 0.15  | 1.28      |
| $8 \times 10^{-7}$   | TA 98   | *     |           |                      |         |       |           |
| $1.6 \times 10^{-7}$ | TA 98   | *     | 0.08      | 0.0001               | TA 1537 | *     | *         |
| $3.2 \times 10^{-8}$ | TA 98   | 0.22  | *         | $2 \times 10^{-5}$   | TA 1537 | *     | *         |
|                      |         |       |           | $2 \times 10^{-6}$   | TA 1537 | *     | *         |
| 0.0001               | TA 100  | *     | *         | $8 \times 10^{-7}$   | TA 1537 | *     | *         |
| $2 \times 10^{-5}$   | TA 100  | *     | 0.14      | $1.6 \times 10^{-7}$ | TA 1537 | *     | 0.13      |
| $4 \times 10^{-6}$   | TA 100  | 0.07  | 0.05      | $3.2 \times 10^{-8}$ | TA 1537 | *     | *         |
| $8 \times 10^{-7}$   | TA 100  | 0.08  | *         |                      |         |       |           |
| $1.6 \times 10^{-7}$ | TA 100  | *     | *         | 0.0001               | TA 1538 | *     | *         |
| $3.2 \times 10^{-8}$ | TA 100  | *     | *         | $2 \times 10^{-5}$   | TA 1538 | *     | *         |
|                      |         |       |           | $4 \times 10^{-6}$   | TA 1538 | *     | *         |
| 0.0001               | TA 1535 | *     | 0.95      | $8 \times 10^{-7}$   | TA 1538 | *     | 0.06      |
| $2 \times 10^{-5}$   | TA 1535 | 0.68  | 1.28      | $1.6 \times 10^{-7}$ | TA 1538 | 8     | *         |
| $4 \times 10^{-6}$   | TA 1535 | 0.23  | 1.06      | $3.2 \times 10^{-8}$ | TA 1538 | *     | 0.23      |

\*Calculated value resulted in a negative MUTAR

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